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Mechanobiology of the Skeleton

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Abstract

Mechanical loading of the skeleton is essential for the development, growth, and maintenance of strong, weight-bearing bones. Bone strength is plastic and can be modulated in adults, as illustrated by the increased bone mass in the playing arms of athletes as compared with their nonplaying arms. Our studies have shown that mechanical loading improves bone strength by inducing bone formation in regions of high strain energy. Therefore, bone tissue has a mechanosensing apparatus that directs osteogenesis to where it is most needed to increase bone strength. The most likely sensors of mechanical loading are the osteocytes, which are visco-elastically coupled to the bone matrix so that their biological response increases with loading rate; thus, increasing loading frequency improves the responsiveness of bone to loading. The osteocyte-specific protein sclerostin, an inhibitor of the Wnt signaling pathway, appears to be one of the mediators of the mechanical loading response. Mechanical loading suppresses osteocyte sclerostin secretion, which allows Wnt signaling-dependent bone formation to occur. Intracellular calcium signaling, adenosine triphosphate signaling, and signaling through second messengers, such as prostaglandins and nitric oxide, precede sclerostin secretion. Stretch-activated ion channels and focal adhesion proteins may play a role in triggering these pathways upstream of sclerostin. In particular, focal adhesion kinase and proline-rich tyrosine kinase 2 appear to be sensors of mechanical loads in bone cells.

Presentation Notes

Slide 1: Science Signaling logo

The slideshow and notes for this presentation are provided by *Science Signaling* (www.sciencesignaling.org).

Slide 2: Mechanobiology of the skeleton

This talk focuses on the mechanisms by which bone tissue adapts to mechanical loading.

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Editor's Note: This contribution is not intended to be equivalent to an original research paper. Note, in particular, that the text and associated slides have not been peer-reviewed.

Slide 3: Types of bone cells

There are several cell types involved in bone development, physiology, and metabolism. These include the osteoblasts that form new bone by secreting the extracellular matrix that is the basis of mineralized bone, the osteoclasts that remove bone, and the osteocytes embedded within the bone matrix (1). Osteocytes have long dendritic processes that contact neighboring cells at gap junctions. Osteocytes detect deformation of the bone matrix caused by mechanical loading and send paracrine signals to osteoblasts and osteoclasts in response. Osteocytes form a network that passes calcium signals from cell to cell, either intracellularly through gap junctions or extracellularly through paracrine signals, such as adenosine triphosphate (ATP). ATP released from one osteocyte binds to P2Y receptors to induce intracellular calcium release in neighboring osteocytes, which creates a self-propagating calcium wave (2).

In general, mechanical loading stimulates the signals for bone formation while also suppressing the activity of the bone-resorbing osteoclasts. If bone enters a state of disuse, the osteocytes send signals that recruit osteoclasts and stimulate bone resorption. The initiation of bone resorption by disuse or bone formation by mechanical loading is called activation.

Slide 4: Bone biology of tennis players

Bone tissue adapts locally to mechanical loads. For instance, tennis players develop larger, denser bones in their playing arms (red arrow) relative to their non-playing arms (3).

Slide 5: Bone biology of baseball players

Like tennis players, baseball players predominantly load one arm. Throwing causes torsion on the humerus, which adapts to this loading by increasing in mass. For example, a college baseball player we examined had greater than 60% more bone mass in his playing arm, as measured by bone mineral content (BMC). Computed tomography analysis revealed this to be the result of increased outer circumference (PeriC) of the bone and decreased inner circumference (EndoC). The torsional rigidity (I_p) of the humerus was almost doubled in the playing arm.

Slide 6: Tennis-playing rodents

Since we study bone remodeling rats and mice, we set out to develop a system we could use to load one forelimb exclusively, like the loading on a tennis player.

Slide 7: Rodent forelimb loading model

The system we use, developed by Torrance *et al.* (4), consists of a machine that applies repeated axial loads to the forelimb. The ulna is curved, so it bows when it is loaded. This bowing causes a predictable pattern of deformation (strain) within the bone tissue (right panel). The greatest strain is compressive and focused on the medial surface (top, in red) of the ulna. The second highest strain value is tensile strain on the lateral surface (bottom, in orange). Between these surfaces is a region of zero strain called the neutral axis. The surfaces with high strain also have high strain energy. This system stimulates new bone formation on the bone surfaces in proportion to the strain energy they receive.

Slide 8: Loading must be dynamic to build bone

We made several important observations using the forelimb loading system. For instance, it is clear that dynamic, cyclical loading induces bone formation and that static, stationary loading does not (5). Shown here are sections of control ulnas (left) next to those that were subjected to loading exercise (right). The exercised ulnas were loaded similarly, but the bone

on top received stationary loading, whereas the bone below received cyclic loading at 2 cycles/s (Hz). Fluorochrome (yellow) labels indicate the bone boundary at the beginning and end of the experiment. A significant amount of new bone was formed under cyclic loading. New bone growth occurred on the medial (top) and lateral (bottom) bone surfaces where the strain energy was highest.

Slide 9: Osteocytes: Embedded bone cells

The reason bone responds only to dynamic loading has to do with the microanatomy around the osteocytes embedded within the bone tissue (6). Osteocytes are regularly spaced within the bone matrix, where they occupy cavities called lacunae and their processes extend through channels called canaliculi.

Slide 10: Osteocytes are mechanosensors

Osteocytes are surrounded by an un-mineralized fluid-filled space within the mineralized bone matrix and are attached to the bone matrix by focal adhesions. In these electron microscopy images, one can see the fluid-filled space between the mineralized matrix and the osteocyte (left). This fluid also fills the space between the osteocyte processes and the lamina limitans (right) (7).

Slide 11: Viscous coupling of osteocytes

As bone is loaded, extracellular fluid is pushed back and forth across the osteocyte membrane. The fluid is of sufficiently high viscosity to exert shear stress on the cell membrane and drag forces on the extracellular proteins that tether the osteocyte to the bone matrix. The magnitude of the fluid force is proportional to the loading rate, so the greater the load rate, the greater the shear stress applied to the osteocytes. Osteocytes are therefore viscously coupled to the bone, and this explains why they are more sensitive to dynamic rather than static loading.

Slide 12: Bone tissue is sensitive to loading frequency

The graph on the left shows dose-response curves for the bones' response to mechanical loading (8). The average bone formation rate is shown on the y axis, and the peak strain engendered in the bone tissue is plotted along the x axis. As the frequency of loading is increased from 1 Hz to 5 Hz to 10 Hz, the slope of each curve increases significantly. Not only is bone added at a greater rate with increased loading frequency, but the amount of loading force required to stimulate bone growth decreases with increased loading frequency. For example, it takes much less bone strain to stimulate bone formation when loading at 10 Hz compared with 5 or 1 Hz. The graph on the right shows the minimum strain needed to initiate bone formation. This value is 1820 microstrain at 1 Hz, but drops to 1180 microstrain at 5 Hz and to only 650 microstrain at 10 Hz.

Slide 13: Model for osteocyte deformation

The microstructure of the osteocyte processes as modeled by Weinbaum and colleagues (9) is shown here. This model predicts that fluid drag forces stretch the extracellular matrix proteins, which creates tension in the cell membrane. This membrane deformation (hoop strain) increases with loading frequency, but plateaus at around 10 Hz, consistent with our experimental findings on loading frequency and bone growth (8, 10).

Slide 14: Engineering a stronger bone

The forelimb loading system I described earlier produces a specific distribution of strain energy within the bone tissue. In the top panel, the highest strain energy is shown in red and the lowest in white. The amount of new bone formed in response to loading is proportional

to the magnitude of the strain energy. A cross section from a rat ulna is shown in the bottom panel. This rat was subjected to forelimb loading 3 times per week for 16 weeks, with each loading session lasting only 3 min. This short loading program substantially changed the shape of the bone section. The red line is a label showing the outline of the bone at the beginning of the experiment. The locations of new bone formation are clear and correspond to the red and orange regions in the top panel. This reshaped bone is about twice as strong as the original bone (11), and the fatigue life of the bone is extended 100-fold (12).

Slide 15: Adaptation of the rat ulna

We measured the adaptation of ulnae from several rats and plotted the changes along the long axis of the bone. We found that the adaptation occurred in a region between 10 and 30 mm from the proximal end of the bone. We then modeled this response computationally.

Slide 16: Computational modeling

We developed a computational model using finite element analysis (FEA) applied to a solid model of a rat ulna generated by micro-computed tomography scans. Bone growth in response to mechanical loading is simulated by allowing the outer surfaces of the model to expand. The growth rate at any point on the bone surface (db/dt) is taken to be the product of an amplification factor (A) and the difference between the magnitude of the local strain energy (ϕ) and its reference threshold value (ϕ_{ref}); thus, $db/dt = A(\phi - \phi_{ref})$. A and ϕ_{ref} are the only model parameters needed to fit the model results to experimental data. We make only two assumptions: First, the osteocytes within the bone can detect the magnitude of strain energy (modeled with ϕ_{ref}), and secondly, the osteocytes will send a signal to osteoblasts on the bone surface that tells them to form bone.

Slide 17: Predicting the cellular response

We found that this simple computational model accurately reproduces the bone shape changes observed in our experiments. The top panels show ulna cross sections before and after growth, with an experimental measurement on the left and results from the computational model on the right. The graph shows the adaptation of the ulna along its axis, in the form of the minimum moment of inertia of the cross section. Computational results are shown in purple, and experimental measurements are shown in black with error bars. Because the results of the model closely matched the experimentally observed results, we conclude that our assumptions about osteocytes are accurate: They detect strain energy and send a signal to osteoblasts. We now believe that signal is a protein called sclerostin.

Slide 18: Sclerostin is constitutively expressed in osteocytes

Sclerostin is produced almost exclusively by osteocytes. It is secreted constitutively, and ~90% of osteocytes are positive for sclerostin as assayed by immunohistochemistry using an antibody specific for sclerostin (13).

Slide 19: Sclerostin is an inhibitor of bone formation

Mice with null mutations in the gene encoding sclerostin (*Sost*) have larger bones and much greater bone mass than wild-type (WT) mice.

Slide 20: Molecular mediators: Wnt signaling

Sclerostin (Sclr in the diagram) is an inhibitor of the Wnt signaling pathway, which is important for bone formation. It binds to Lrp receptors and blocks Wnt binding and thus blocks Wnt signaling. Other inhibitors of Wnt signaling are Dickkopf 1 (Dkk1) and secreted frizzled-related protein 1 (sFrp1) (14).

Slide 21: Mechanical loading and Wnt pathway inhibitors

We examined the effects of mechanical loading on the abundance of Sost, Dkk1, and sFrp1 in bone tissue. Loading applied to forelimbs of mice substantially decreased Sost and Dkk1 expression, but sFrp1 expression was unchanged (15), as measured by quantitative polymerase chain reaction.

Slide 22: Unloading and Wnt pathway inhibitors

Unloading the hind limbs of mice using the tail suspension method increased the amount of Sost in the bone tissue, but had no effect on Dkk1 or sFrp1 abundance (15). These findings suggest that production of both Sost and Dkk1 are sensitive to mechanical loading, whereas only Sost abundance is affected by unloading.

Slide 23: Sclerostin is a strain-sensitive protein

We then examined sclerostin protein by immunohistochemistry (IHC) in mouse ulnae after fore limb loading. We found that sclerostin levels decreased substantially within 24 hours after loading. The top right shows osteocytes stained to show sclerostin, but staining almost disappears after loading (bottom right) (15).

Slide 24: Sclerostin and mechanical strain

We found that the decrease in osteocytes expressing sclerostin protein was proportional to the strain (or strain energy) induced in the bone tissue. As we showed earlier, the strain forces vary along the axis of the ulna. Sclerostin abundance decreased by almost a factor of four at the distal (rightmost) region of the ulna, where the strains were largest, as compared with the proximal (leftmost) region (15).

Slide 25: Change in sclerostin is proportional to strain energy

Changes in sclerostin abundance in the bone cross section were directly proportional to strain energy within the bone tissue. We found that the number of osteocytes that stained positive for sclerostin decreased by almost 80% in the medial (MED) region of the bone in response to loading. There was no significant drop in sclerostin along the neutral axis (NA) of the bone, but the number of sclerostin-positive osteocytes decreased by 30% in the lateral (LAT) region (15). Strain energy was linearly correlated with changes in sclerostin abundance (lower right).

Slide 26: Sclerostin summary

Osteocytes constitutively secrete sclerostin, which inhibits bone formation by blocking Wnt signaling in osteoblasts on the bone surface. Mechanical loading suppresses sclerostin secretion, which allows osteoblasts to form new bone.

Slide 27: Molecular mediators: Wnt co-receptor Lrp5

We investigated the mechanism of sclerostin action and determined that sclerostin inhibits Wnt signaling by binding to the Wnt coreceptor Lrp5 and the related protein Lrp6.

Slide 28: Lrp5 is necessary for normal bone development

As compared with WT, mice homozygous for a null mutation in *Lrp5* have osteoporotic bones because of decreased bone formation (16).

Slide 29: *Lrp5* KO mice do not respond properly to forelimb loading

Forelimb loading induces new bone formation in the WT ulna, illustrated by the red and green labels (top right). In *Lrp5* knockout (KO) mice, these labels are close together, which shows little stimulation of bone formation (bottom right) (16).

Slide 30: Bone response to mechanical loading is suppressed in *Lrp5*-deficient mice

When we quantified the bone formation response, we found that bone formation was suppressed by 88% in male *Lrp5* KO mice and 99% in female *Lrp5* KO mice. The graphs show dose-response curves for new bone formation (*y* axis) induced by various amounts of forelimb loading (*x* axis) in WT (WT, blue), *Lrp5*^{+/-} (HET, green), and *Lrp5*^{-/-} (KO, red) mice. The slopes of these curves represent the responsiveness of the bone to mechanical loading (16).

Slide 31: GFP labeling of osteoblasts

Our next goal was to determine what happened to osteoblasts after forelimb loading in *Lrp5* KO mice. To visualize osteoblasts, we generated mice that carry a transgene that encodes green fluorescent protein (GFP) under the control of promoters that are activated at different stages of osteoblast differentiation. Many stage-specific osteoblast promoters have been identified (top panel), two of which we used to build stage-specific osteoblast reporter constructs: the 3.6-kb fragment of the *Col1a1* promoter (Col3.6), which is active in early osteoblasts, and the 2.3-kb fragment of the *Col1a1* promoter (Col2.3), which is active in mature osteoblasts and in osteocytes. In our experiments, we used the Col2.3 and Col3.6 promoters to drive expression of *GFP* in osteoblasts.

Slide 32: Osteoblasts are recruited normally in *Lrp5* KO mice

These panels show our results using the Col3.6 promoter to label osteoblasts. In each row, the leftmost panel shows the GFP labeled osteoblasts that are recruited to the bone surface after forelimb loading. There is no difference in number of recruited osteoblasts between WT and *Lrp5* KO mice. (K) and (L) show numbers of labeled osteoblasts counted by two different methods. However, osteoblasts from the *Lrp5* KO mice did not form new bone in response to forelimb loading (16). Therefore, we conclude that recruitment of osteoblasts in response to mechanical loading was normal in *Lrp5* KO mice, but those osteoblasts were not able to form new bone without *Lrp5*. This suggests that the early steps of mechanotransduction, the processes required to sense and respond to mechanical force, are intact in these KO mice.

Slide 33: Mechanotransduction timeline

Mechanotransduction in bone requires numerous steps that occur at various times after the mechanical stimulus. Early events that occur within a minute include ATP release and intracellular calcium (iCa²⁺) signaling. These are followed by second messengers, including prostaglandins (PGE₂), nitric oxide (NO), and signaling through mitogen-activated protein kinases (MAPKs), such as the extracellular signal-regulated kinase (ERK). Wnt signaling, to which *Lrp5* and sclerostin contribute, occurs fairly late in the mechanotransduction cascade. An important unanswered question is what initiates this signaling cascade?

Slide 34: What is the mechanosensor?

Several mechanosensors in bone cells have been proposed, including stretch- and voltage-sensitive calcium channels (VSCC), focal adhesion proteins like focal adhesion kinase (FAK), and proline-rich tyrosine kinase 2 (Pyk2) that are linked to the extracellular matrix by integrins, and a G protein-coupled receptor (GPCR).

Slide 35: Model involving FAK and Pyk2

We have found evidence for stretch-activated calcium channels and focal adhesion proteins acting as mechanosensors. These two mechanisms may cooperate, given that intracellular calcium might reinforce signaling at focal adhesions by activating Pyk2, a calcium-sensitive kinase that binds to FAK. Downstream signaling may include MAPK pathways (17).

Slide 36: FAK-mediated mechanotransduction in skeletal regeneration

There is emerging evidence that FAK plays a role in bone mechanotransduction. In particular, Jill Helms's group has shown that bone regeneration around a mechanically loaded implant is suppressed in FAK-deficient mice (18). This study used mice in which the gene encoding FAK was knocked out specifically in osteoblasts using the Cre-lox method (lower right panels). A small pneumatic plunger was implanted in a long bone (panels A and B), and loading was applied to the healing bone. The left panels show the bone response in WT and KO mice. Panel E shows new bone (dark blue) around the plunger in WT mice. No new bone was observed in FAK-deficient mice (panel G).

Slide 37: Pyk2 KO mice have high bone mass

An important role for Pyk2 in bone biology was shown recently by Buckbinder and colleagues (19). *Pyk2* KO mice have high bone mass resulting from increased bone formation (left, panel H). Another group has shown that these mice also have decreased bone resorption (right, panels A and B) (20).

Slide 38: Pyk2 regulates osteocyte apoptosis

Mechanical loading suppresses osteocyte apoptosis, whereas unloading induces osteocyte apoptosis (21, 22). Therefore, mechanisms of osteocyte apoptosis are probably important in mechanotransduction. Plotkin and colleagues (23) have shown that dexamethasone induces osteocyte apoptosis by activating Pyk2 (upper left panel), and the Pyk2 replacement of Tyr⁴⁰² with Phe (Y402F) mutation blocks this effect (upper right panel). Calcium chelating agents, such as BAPTA and EG-TA, and gadolinium, a blocker of stretch-activated channels (lower panels) inhibits osteocyte apoptosis in response to dexamethasone. These findings indicate that Pyk2 must be activated to initiate osteocyte apoptosis and that calcium is necessary to activate Pyk2 in osteocytes. Furthermore, it appears that stretch-activated channels may be involved. Although the role of Pyk2 in osteocyte mechanotransduction is unknown, these results suggest its involvement.

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